Pituitary, Neuroendocrinology and Puberty Session 1

FC8.1

Hypothalamic AgRP Neurons Drive Endurance in Food-restricted Mice

Maria Consolata Miletta, Tamas L. Horvath

Yale University School of Medicine, New Haven, USA

Diseases of food restriction, such as anorexia and bulimia nervosa, are psychiatric conditions with the highest mortality. It is not known how these disorders emerge and what determine mortality. Individuals with these disorders frequently engage in compulsive exercise. States of food restriction are associated with elevated activity of hypothalamic neurons that produce AgRP, which cells are crucial for feeding and can promote stereotypic behaviors.

Here, we interrogated whether these hypothalamic neurons are involved in sustained compulsive exercise during food restriction. Using a combined pharmacologic and genetic approach, we found that regardless of AgRP circuit activity, food-restricted animals engaged in compulsive exercise if a running wheel was available, but there was a positive correlation between AgRP circuit activity and exercise volume. Strikingly, animals with impaired AgRP circuitry died of exhaustion after few days of compulsive running, while those animals, in which we activated AgRP neurons daily, had significantly increased endurance of compulsive exercise compared to all other groups without lethality during the trial.

As a mechanistic cause of the involvement of AgRP neurons in endurance exercise, we found that these cells are crucial for proper mobilization of lipids from fat stores, a known determinant of endurance running.

These observations shed new light on a previously unsuspected organizational role of AgRP neurons in the regulation and dysregulation of complex behaviors via both neuronal and systemic actions with direct implications for psychiatric conditions such as anorexia nervosa.

FC8.2

Analysis of Hypothalamic Metabolic Circuits after Normalization of Body Weight in Mice that had been Obese due to high fat diet intake

Santiago Guerra-Cantera [1,2,3], *Laura Frago* [1,2,3], *Purificación Ros* [2,4], *Sandra Canelles* [1,3], *Francisca Díaz* [1,3], *Alejandra Freire-Regatillo* [1,2,3], *Marta Marta Torrecilla-Parra* [1,2], *Jesús Argente* [1,2,3,5], *Julie A. Chowen* [1,3,5]

[1]Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación La Princesa, Madrid, Spain. [2]Department of Pediatrics, Universidad Autónoma of Madrid, Madrid, Spain. [3]Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain. [4]Department of Pediatrics, Hospital Universitario Puerta de Hierro-Majadahonda, Madrid, Spain. [5]IMDEA Food Institute, CEI UAM + CSIC, Madrid, Spain

The obesity epidemic continues to be a dramatic problem in the developed world despite attempts to curtail its rise. Reducing energy intake and/or increasing energy expenditure can result in weight loss; however, if one returns to their poor lifestyle habits the previous weight is not only recuperated, but often surpassed.

We hypothesized that although a normal body weight may be achieved, the hypothalamic circuits controlling appetite and energy expenditure may not return to normal, at least in the same time-frame.

To this end, male and female 7-week old C57/BL6J mice were fed a high fat diet (HFD; 60% kcal from fat, 20% kcal from carbohydrates, 5.1 kcal/g) or standard rodent chow (3.1 % kcal from fat, 76% kcal from carbohydrates, 3.41 kcal/g) for 2 months. Then, half of the HFD group was returned to the normal chow diet (HFDCH). All mice were killed one month later, with a glucose tolerance test (GTT) being performed one week before. Hypothalmi were processed for real time PCR. At two months all HFD mice had gained significantly more weight than the chow mice. After the return to chow, HFDCH mice lost weight and after one month their weight was not different from chow mice. Although HFD increased fasting glucose in both sexes, only male HFD mice had an increased area under the curve in the GTT ($p<0.001$). Fasting glycemia and energy intake of HFDCH mice normalized one month after the diet changed. Females had higher hypothalamic mRNA levels of neuropeptide Y (NPY) and Agouti-related peptide (AgRP) than males (both $p<0.0002$), with HFD decreasing the expression of these neuropeptides in both sexes. The change from HFD to chow increased AgRP expression to control levels in both sexes. In females NPY mRNA returned to control levels, but in males NPY expression was only partially normalized. Proopiomelanocortin (POMC) mRNA levels were higher in males than females and decreased on the HFD only in males ($p<0.004$) and remained reduced one month after being changed to chow. In females there were no differences in POMC mRNA levels between dietary groups.

In conclusion, there is a clear sex difference in the response of hypothalamic metabolic neuropeptides to dietary changes. Although a normal weight is obtained, the hypothalamic metabolic control system, especially in males, remains altered. Thus, this could result in a more dramatic increase in weight gain if returned to a less healthy lifestyle.

FC8.3

Absence of central adrenal insufficiency in adults with Prader-Willi syndrome

Anna Rosenberg[1,2], *Kirsten Davidse*[1,2], *Karlijn Pellikaan*[1,2], *Stephany Donze*[3,2], *Anita Hokken-Koelega*[4,5,3,2], *Aart Jan van der Lely*[1], *Laura de Graaff*[1,5,2]

[1]Department of Internal Medicine-Endocrinology, Erasmus University Medical Center, Rotterdam, Netherlands. [2]Dutch Center of Reference for Prader-Willi Syndrome, Rotterdam, Netherlands. [3]Dutch Growth Research Foundation, Rotterdam, Netherlands. [4]Department of Pediatric Endocrinology, Erasmus University Medical Center, Rotterdam, Netherlands. [5]Academic Center for Growth, Erasmus University Medical Center, Rotterdam, Netherlands

Introduction: Individuals with Prader-Willi syndrome (PWS) suffer from hyperphagia, hypotonia and hypothalamic dysfunction, leading to a variety of pituitary hormone deficiencies. Central adrenal insufficiency (CAI) has been reported in PWS, while each of these studies used different testing modalities and cut-off values. Therefore, reported prevalence of CAI ranges from 0% to 60%. It has been speculated that CAI might be responsible, at least in part, for the high mortality (3%) in patients with PWS. If CAI is present, timely diagnosis and treatment is needed in order to prevent avoidable mortality. There are no guidelines on the appropriate evaluation and management of CAI in adults with PWS. In our center, many adult patients with PWS receive standard hydrocortisone (HC) treatment around physical and/or psychological periods of stress. Frequent administration of HC increases the risk of obesity, hypertension, osteoporosis and diabetes, already a major problem in adults with PWS. It is therefore of utmost importance to assess the real prevalence of CAI in order to prevent both under- and overtreatment with HC.

Methods: We performed multiple dose metyrapone (MTP) test in 42 patients and insulin tolerance test (ITT) in 9 patients. When levels of 11-DOC during MTP were greater than 230 nmol/L (7.6 g/dL) or levels of cortisol during ITT were greater than 500 nmol/L (18.1 μg/dL), adrenal insufficiency was excluded.

Results: 51 adult subjects (31 males and 20 females), median (range) age 29.2 (18.9 – 58.3) yrs, with genetically confirmed PWS, participated in the study. 22 subjects (43%) were using GH treatment since childhood. Using the MTP or ITT, CAI was excluded in all subjects. Even patients with a low baseline cortisol level (lowest: 119.0 nmol/L) appeared to have a normal MTP/ITT test result. MTP test/ITT were tolerated well by all individuals. Additional revision of medical files of all PWS adults visiting our ternary referral center (n = 120) revealed that none of the patients who underwent surgery without peri-operative hydrocortisone treatment had suffered complications due to hypocortisolism.

Conclusion: Central adrenal insufficiency appeared to be absent in all 51 adults with Prader-Willi syndrome tested by multiple dose metyrapone test or an insulin tolerance test. This indicates that CAI is rare in adults with PWS. Based on these results, we recommend performing a MTP or ITT test before prescribing hydrocortisone medication during periods of psychological or physical stress in all adults with PWS.

FC8.4

Peripheral and Hypothalamic Alterations in The Insulin-Like Growth Factor (Igf) System in Response to High Fat Diet-Induced Weight Gain

Santiago Guerra-Cantera[1,2,3], *Francisca Díaz*[1,3], *Purificación Ros*[2,4], *Alejandra Freire-Regatillo*[1,2,3], *Marta Torrecilla-Parra*[1,2], *Vicente Barrios*[1,3], *Laura Frago*[1,2,3], *Julie A. Chowen*[1,3,5], *Jesús Argente*[1,2,3,5]

[1]Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación La Princesa, Madrid, Spain. [2]Department of Pediatrics, Universidad Autónoma of Madrid, Marid, Spain. [3]Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain. [4]Hospital Universitario Puerta de Hierro-Majadahonda, Madrid, Spain. [5] IMDEA Food Institute, CEI UAM + CSIC, Madrid, Spain

The insulin-like growth factor (IGF) system is fundamental for physiological processes such as growth and metabolism. In addition, in the brain it regulates glucose metabolism and neuroprotection. The IGF axis can be altered by nutritional status, but little is known regarding the effects of specific dietary components on this system.

Our aim was to examine how high-fat diet (HFD) and low-fat/high sucrose diet (LFHSD) intake affect the central and circulating IGF systems.

Male and female 7-week old C57/BL6J mice were fed a HFD (60% kcal from fat, 20% kcal from carbohydrates, 8.9% of weight from sucrose, 5.1 kcal/g), LFHSD (10% kcal from fat, 72% kcal from carbohydrates, 33.1% of weight from sucrose, 3.76 kcal/g) or standard rodent chow (3.1 % kcal from fat, 76% kcal from carbohydrates, 0.9% of weight from sucrose, 3.41 kcal/g) for 2 months. A glucose tolerance test (GTT) was performed a week before sacrifice. Plasma hormone levels were assayed by ELISA and relative gene expression by RT-PCR.

HFD increased weight gain and visceral and subcutaneous adipose tissue levels in both sexes ($p<0.001$) compared to chow and LFHSD. Energy intake was higher on the HFD in both sexes, reaching significance in females ($p<0.001$). Glucose tolerance was impaired only in males on the HFD ($p<0.01$). Plasma levels of free ($p<0.001$) and total ($p<0.001$) IGF1 were higher in HFD mice of both sexes, with HFD also increasing insulin ($p<0.05$) and IGFBP3 ($p<0.01$) levels. HOMA-IR was impaired by HFD in both sexes ($p<0.05$).

In the hypothalamus, IGF1 mRNA levels were increased after HFD consumption ($p<0.05$) in both sexes and by LFHSD only in females ($p<0.05$). Also in females, IGF2 ($p<0.05$) and IGFBP2 ($p<0.01$) mRNA were increased by HFD consumption compared to both chow and LFHSD. In all mice, relative IGF2 and IGFBP2 were positively correlated ($r=0.843$, $p<0.001$). In males, IGFBP5 mRNA levels increased in LFHSD and HFD compared to chow ($p<0.01$). No changes in other members of the IGF family were observed.

In conclusion, the central and peripheral IGF systems are modulated in HFD-induced weight gain, with this effect differing between males and females. In females, the HFD-induced increase in